THE EMBRYOLOGY AND RELATIONSHIPS OF *RHYNCHOCALYX* OLIV. (RHYNCHOCALYCACEAE)[1]

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ABSTRACT

This paper presents the first embryological study of *Rhynchocalyx,* a unique and problematical monotypic South African genus. The genus agrees well with other Myrtales in its basic embryological and other characteristics, including an ephemeral anther endothecium, a micropyle formed by the inner integument alone, and by other subsidiary features of the anther, nucellus, endosperm, and seed, as well as its megasporogenesis. *Rhynchocalyx* differs absolutely from *Axinandra* in many embryological characteristics, a fact that is not in agreement with a broad definition of Crypteroniaceae to include both genera. Also, *Rhynchocalyx,* despite its shared distinctive multicelled archesporium, differs from Lythraceae in many attributes. Thus, evidence from embryology, combined with that from other sources, supports the conclusion that *Rhynchocalyx* is not directly related to Lythraceae, and is, therefore, best assigned to a family of its own, Rhynchocalycaceae.

This paper reports the embryology of the rare monotypic South African genus *Rhynchocalyx,* and is the second concerning the unique genera of the order Myrtales. The first concerned *Axinandra* (Tobe & Raven, 1983b). The proper taxonomic assignment and relationships of *Rhynchocalyx,* like those of *Axinandra,* have often been disputed. Oliver (1895), who first described *Rhynchocalyx lawsonioides,* classified the genus under Lythraceae. Later, Engler (1900) treated *Rhynchocalyx* as one of the "Gattungen der Lythraceen von unsicherer Stellung," and Koehne (1903) excluded it from Lythraceae. Sprague and Metcalfe (1937), however, returned it to Lythraceae, stating that Koehne's grounds for its exclusion from that family were not tenable. Recently, van Beusekom-Osinga and van Beusekom (1975) proposed a broad definition of Crypteroniaceae that included *Rhynchocalyx,* together with *Crypteronia, Dactylocladus, Axinandra,* and *Alzatea.* Pollen morphology might or might not be taken to support a relationship of *Rhynchocalyx* with the other genera of Crypteroniaceae sensu lato (Muller, 1975): thus, *Rhynchocalyx* agrees with *Dactylocladus* and *Axinandra* in having heterocolpate pollen grains but differs from both *Crypteronia* (with bisyncolporate pollen grains) and *Alzatea* (with tricolporate pollen grains). *Rhynchocalyx* and *Alzatea* differ from the other three genera of Crypteroniaceae sensu lato in their wood anatomy, and share many characteristics with Lythraceae and Melastomataceae (van Vliet, 1975).

On the other hand, leaf, twig, and nodal anatomy suggests that *Rhynchocalyx* is closer to some Lythraceae, Oliniaceae, and Melastomataceae than to the other members of Crypteroniaceae sensu lato (van Vliet & Baas, 1975). Nevertheless, differences in floral structure and the presence of foliar sclereids in the petioles of *Rhynchocalyx* (Rao & Das, 1979) indicate that it is not directly related to Lythraceae. In addition, *Rhynchocalyx* may be distinguishable from Lythraceae in not having nectaries in its flowers, although this feature needs to be reviewed in Lythraceae.

Dahlgren and Thorne (1984) pointed out that *Rhynchocalyx* stood apart from Lythraceae in having petals and stamens on the hypanthial rim and sclereids in its leaf petioles. Taking all of these facts into account in their cladistic analysis, Johnson and Briggs (1984) concluded that *Rhynchocalyx* is not directly related to Lythraceae. This conclusion led them to describe the new family Rhynchocalycaceae for the genus, a family that is accepted both by Dahlgren and Thorne (1984) and by Graham (1984), who described a second new family, Alzateaceae, to accommo-

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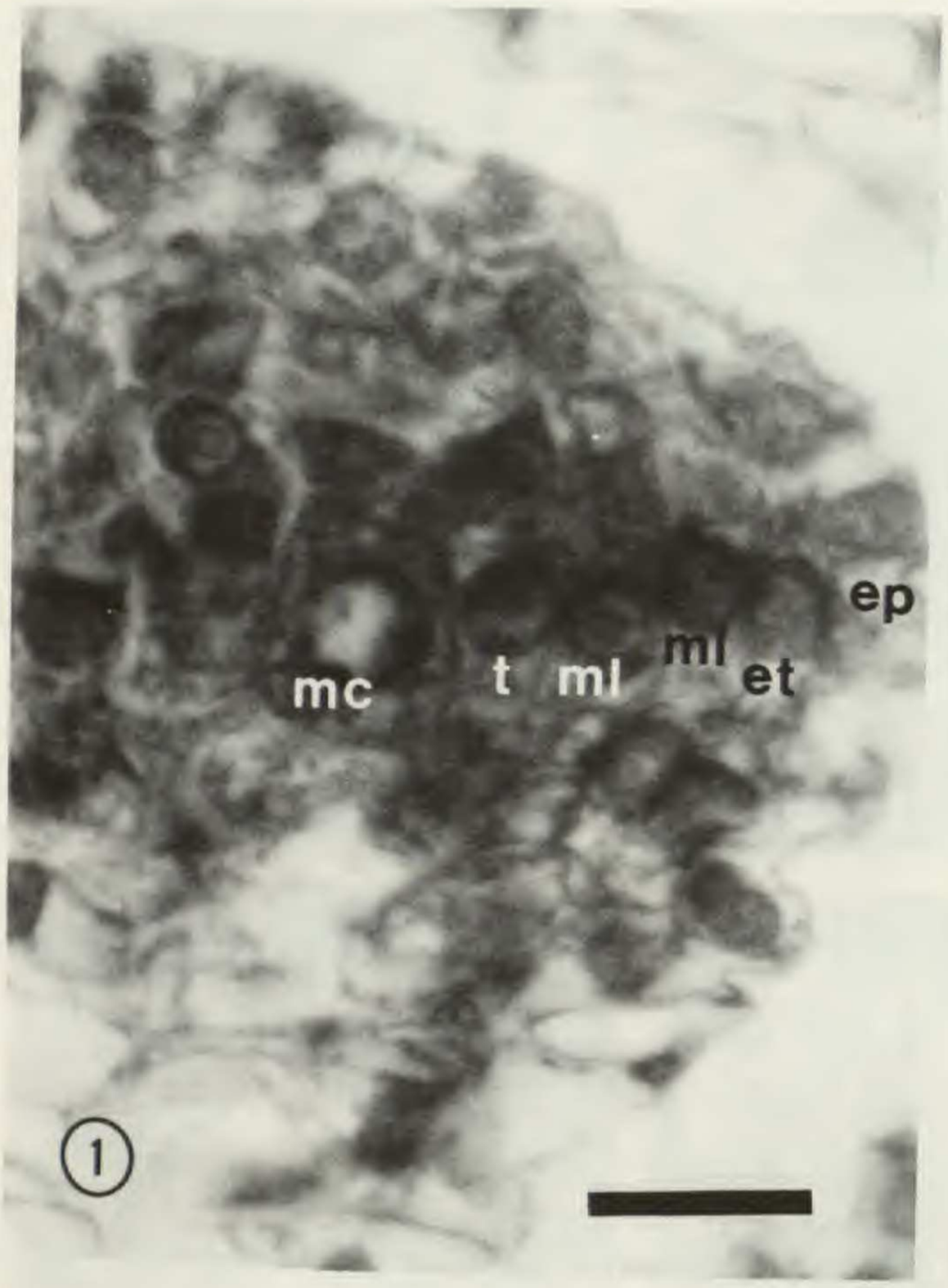
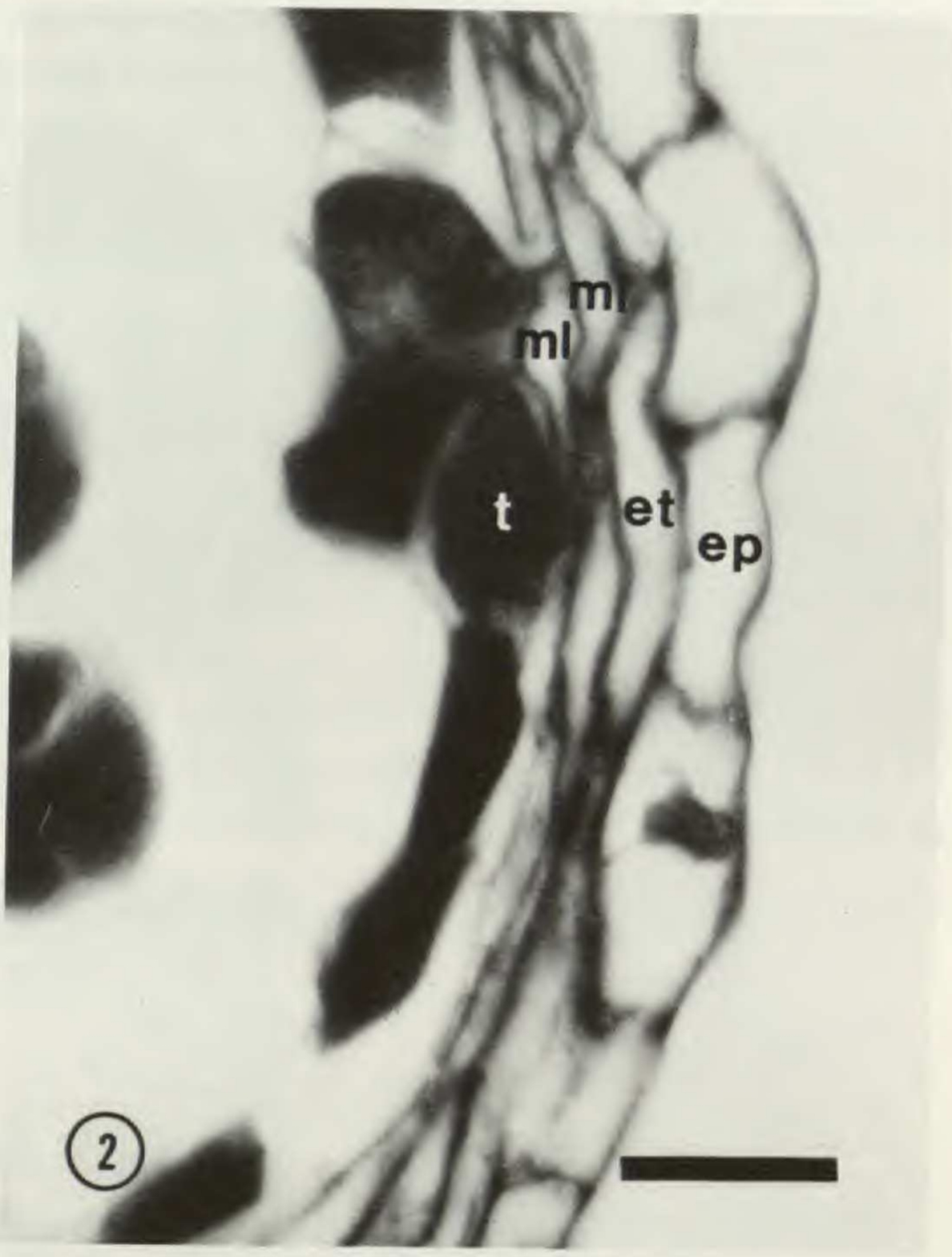
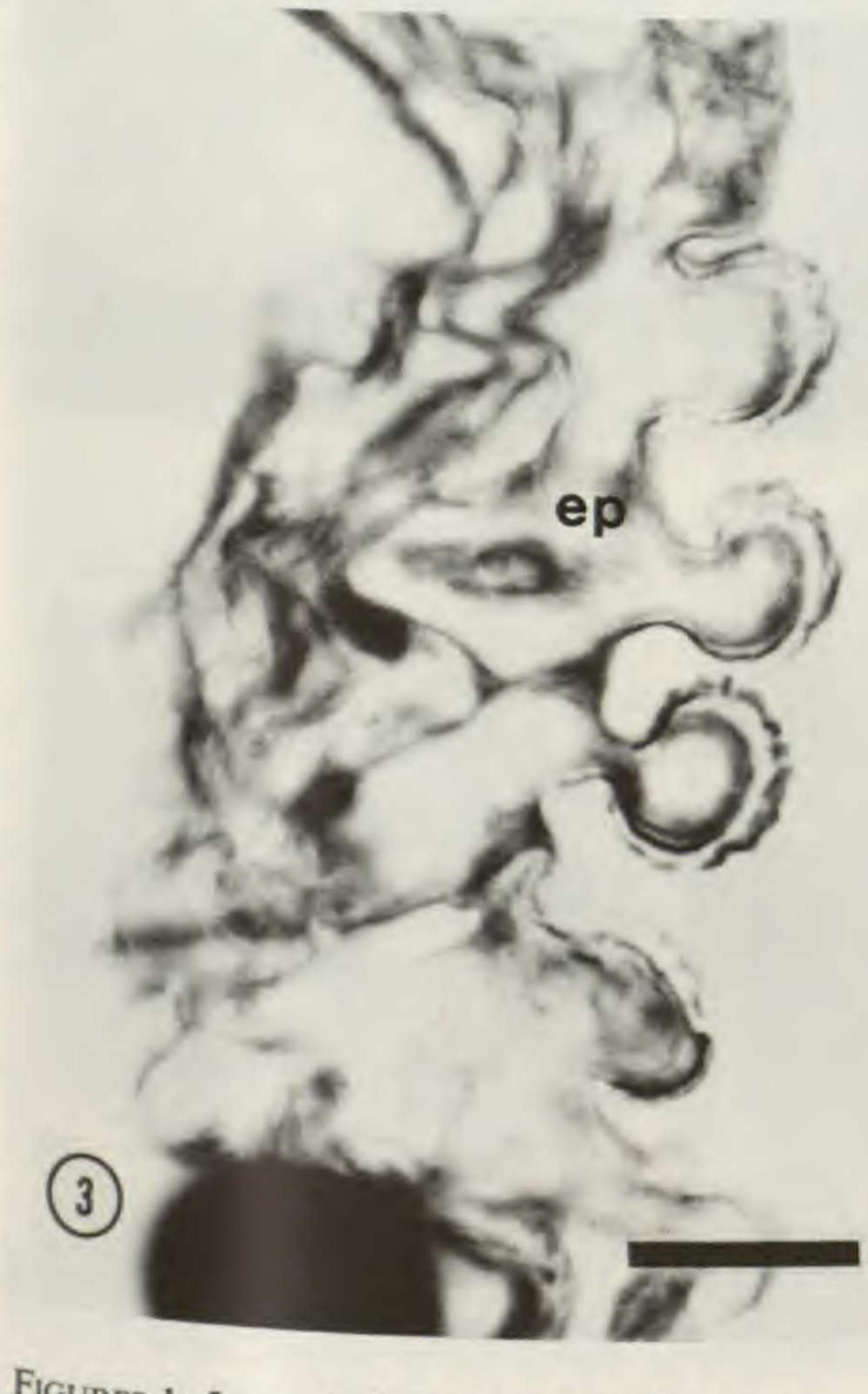
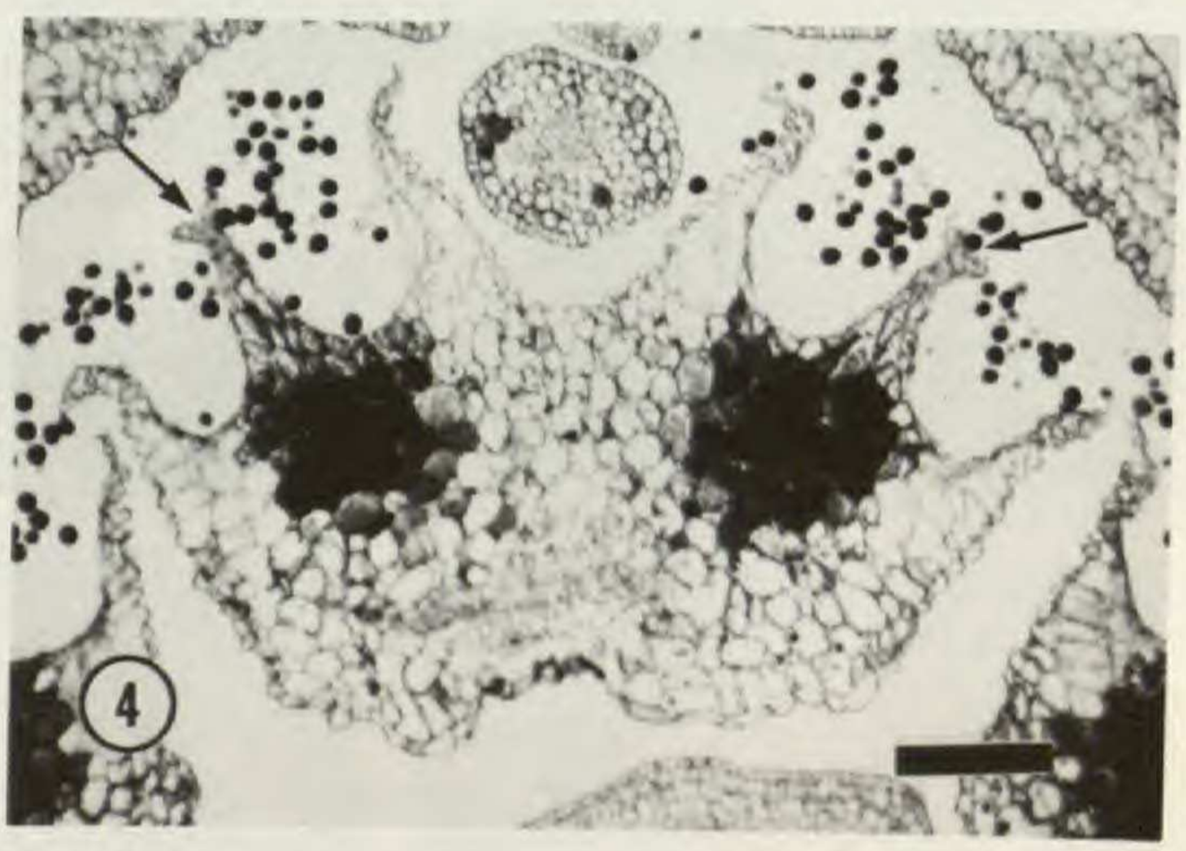
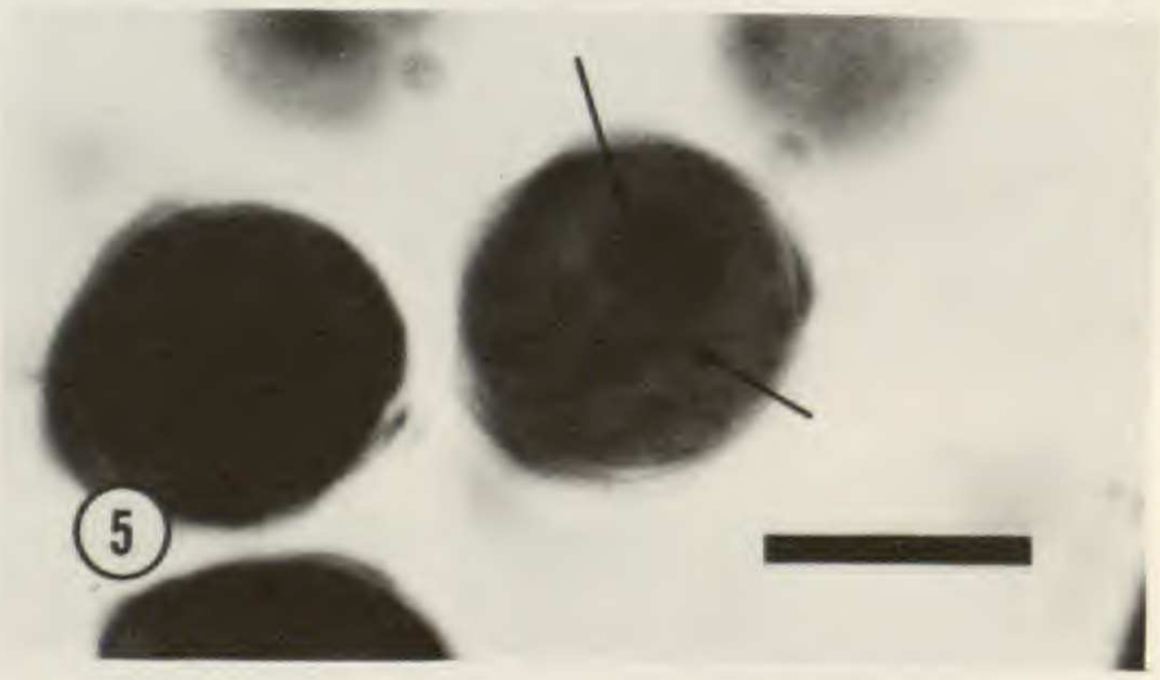


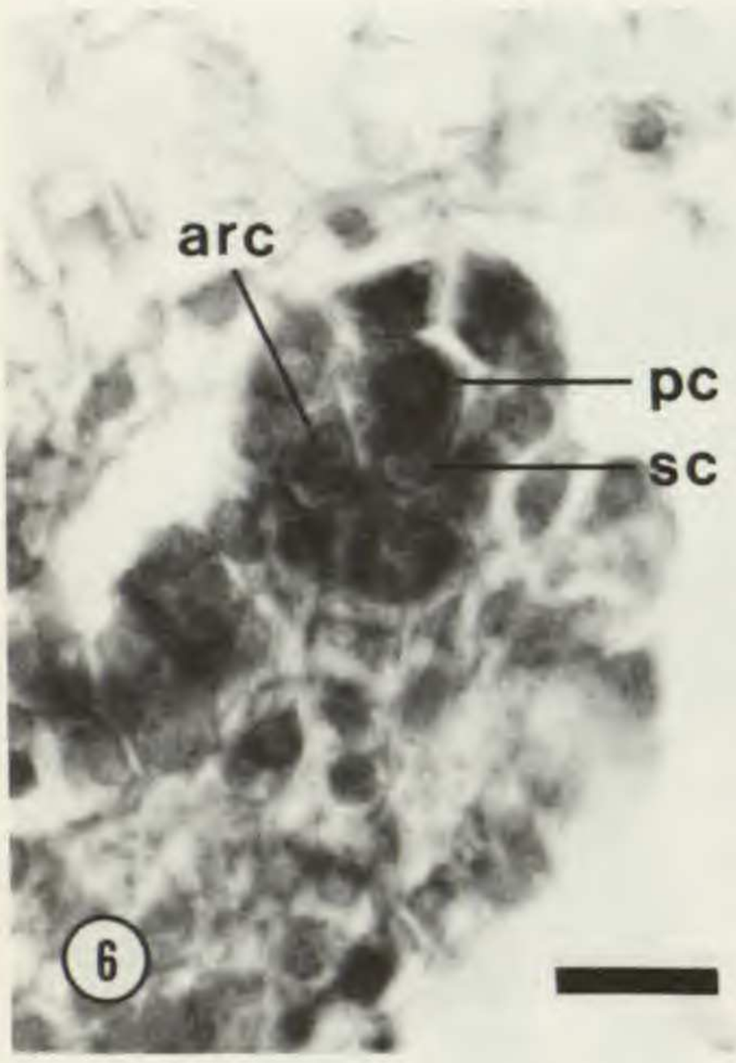


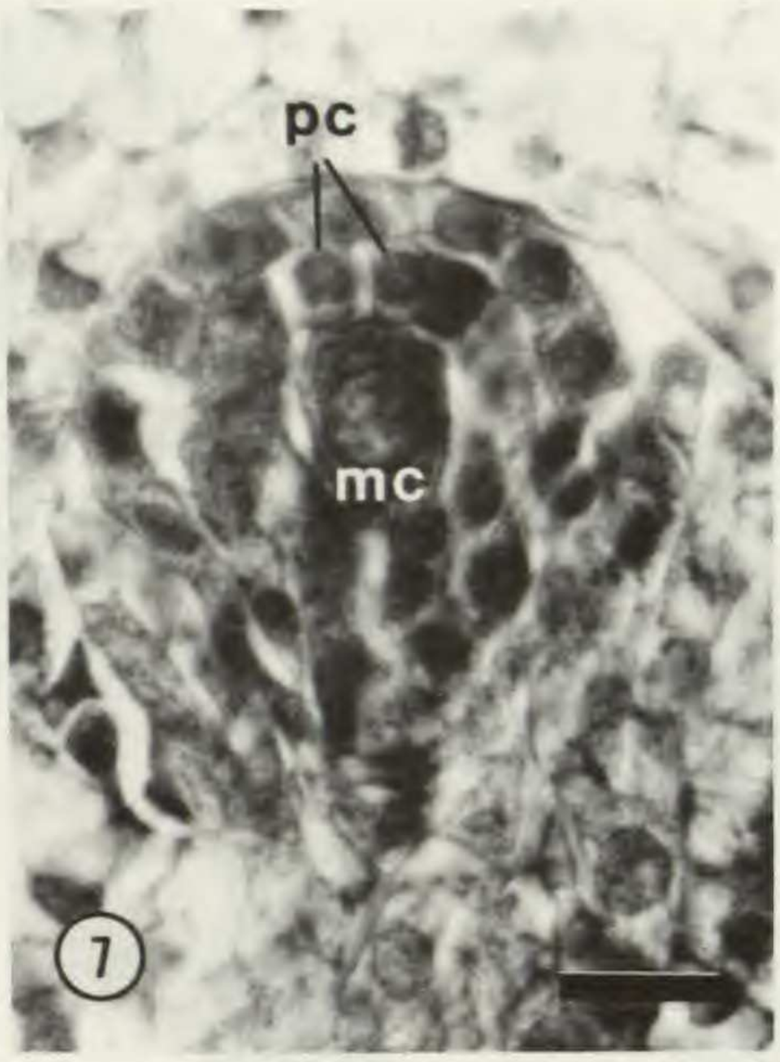


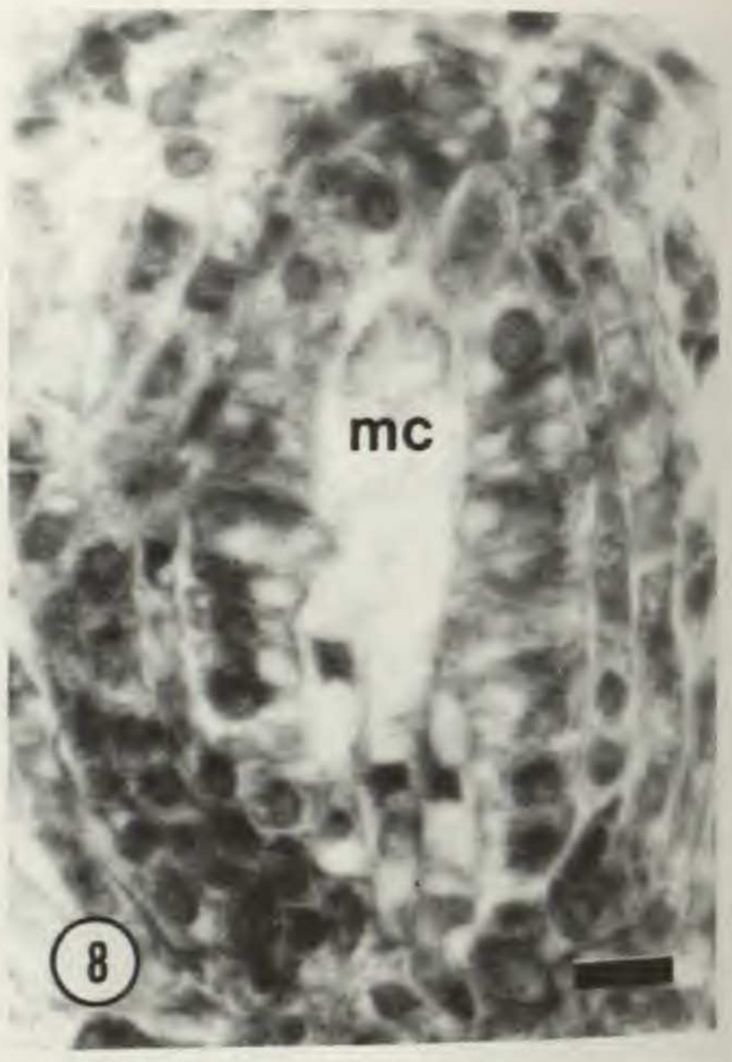


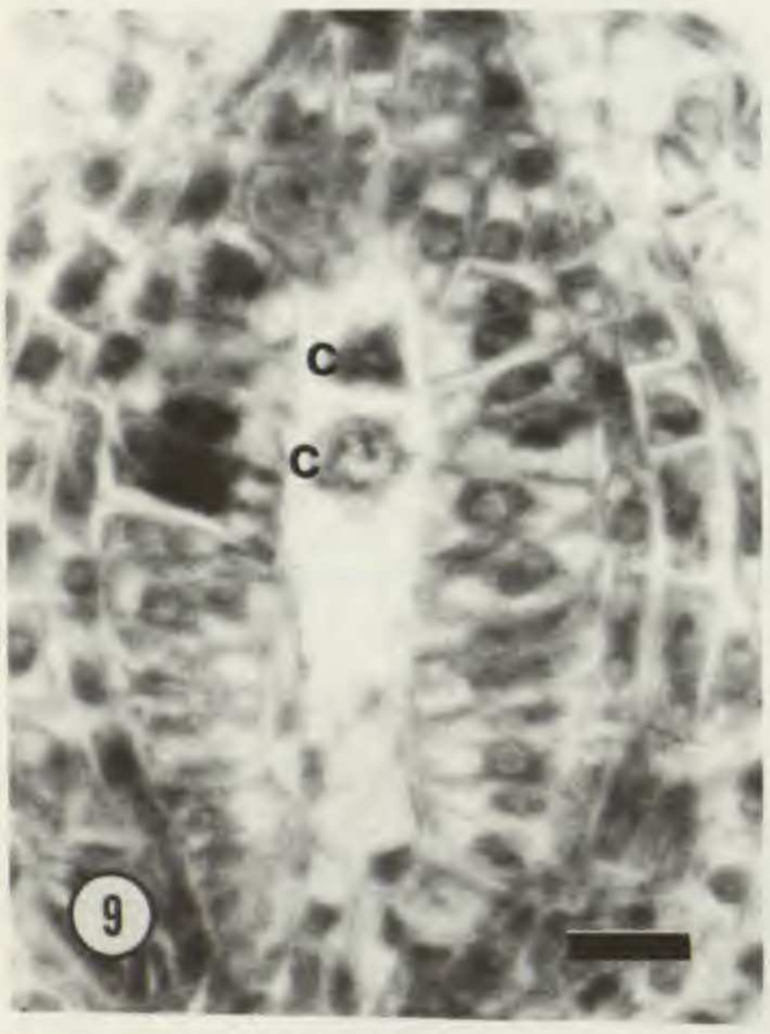


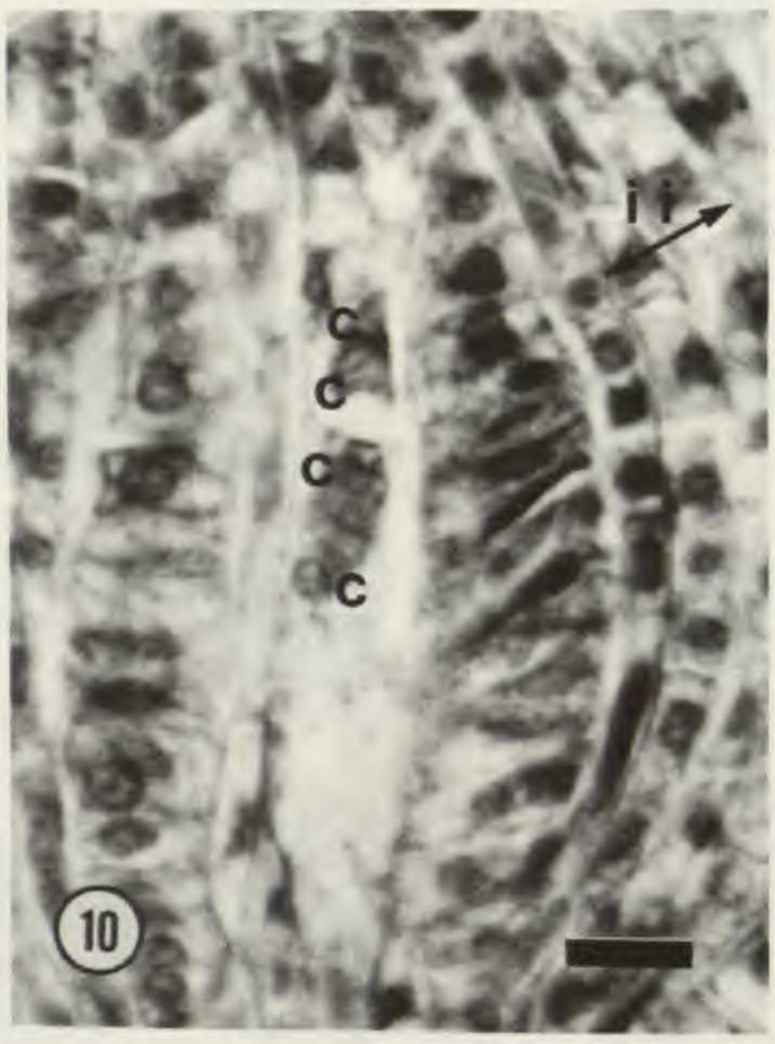
FIGURES 1–5. —1. Cross section of a young anther. Its wall is formed by an epidermis (*ep*), an endothecium (*et*), middle layers (*ml*), and a tapetum (*t*). *mc*: microspore mother cell. Bar = 10 μm.—2. Cross section of an older anther. Only the epidermis (*ep*) remains uncrushed while the endothecium (*et*), middle layers (*ml*) and the tapetum (*t*) are degenerating. Bar = 10 μm.—3. Cross section of a mature anther. Note persistent papillate epidermal cells (*ep*). Bar = 10 μm.—4. Cross section of a dehisced anther. Note the persistent septa (arrows) between two microsporangia on each side of the anther. Bar = 100 μm.—5. Two-celled pollen grain at the time of shedding. Arrows point out a nucleus of a generative cell and that of a vegetative cell. Bar = 10 μm.

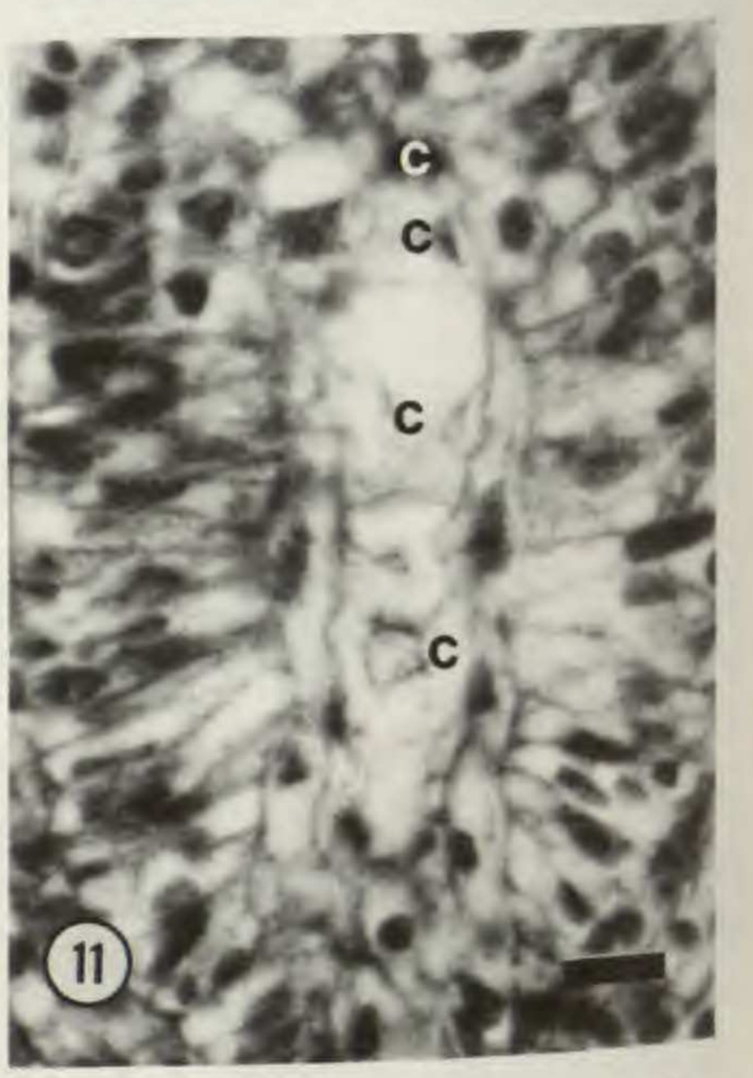


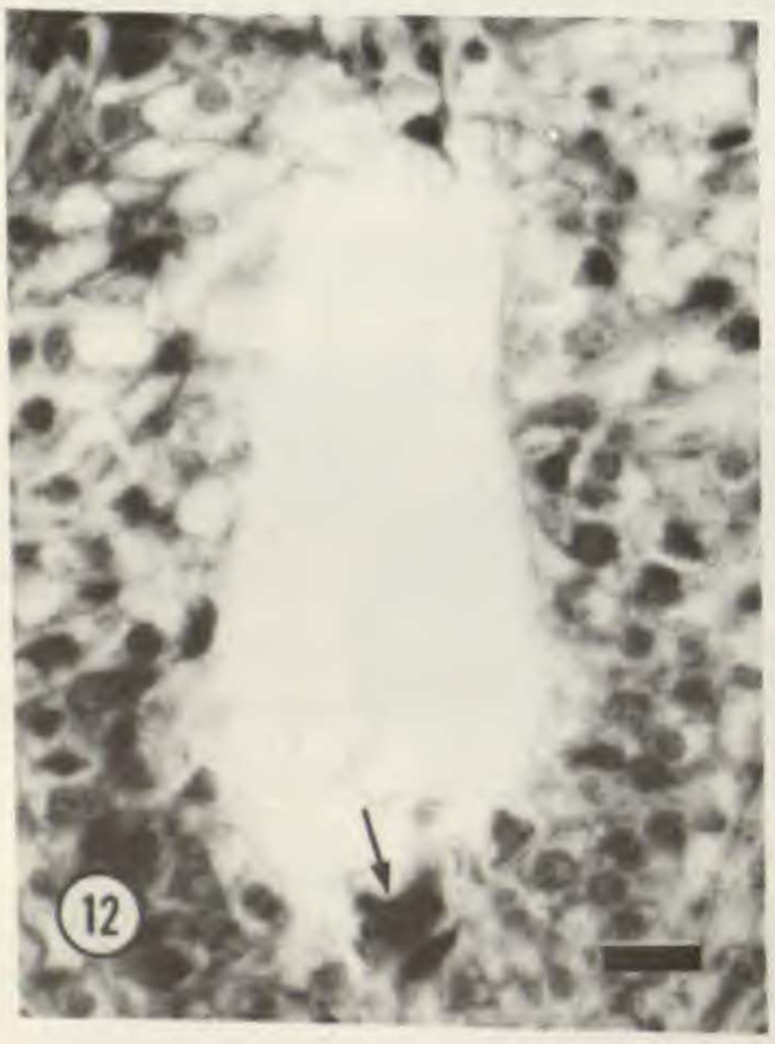


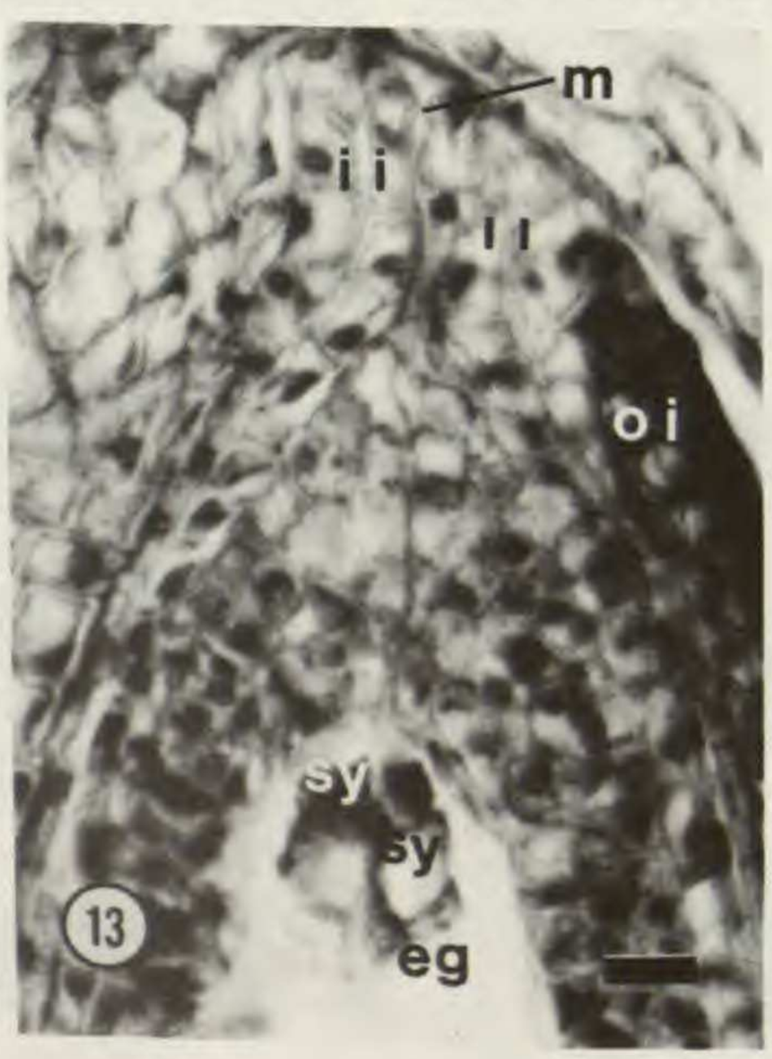


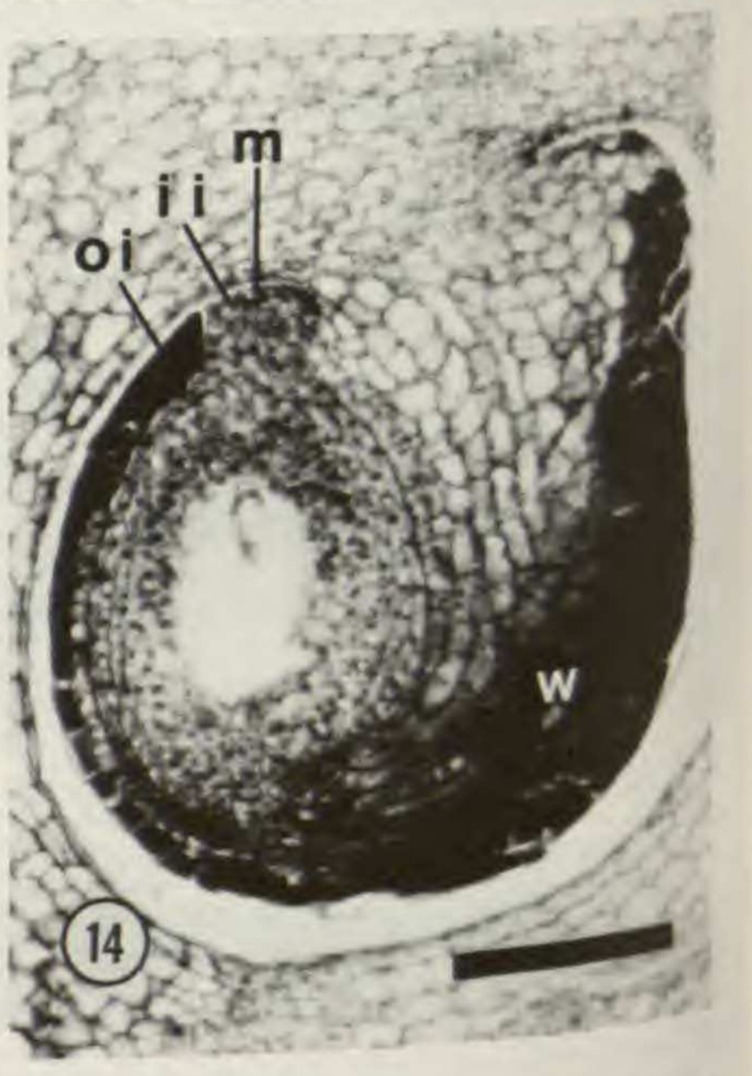












FIGURES 6–14. —6. Longitudinal section of an ovule primordium with a multicelled archesporium. The only functional archesporial cell is already divided into a primary parietal cell (*pc*) and a sporogenous cell (*sc*). The rest of the archesporial cell (*arc*) remains undivided. Bar = 10 μm.—7. Longitudinal section of a young ovule with a young megaspore mother cell (*mc*) below parietal cells (*pc*). Bar = 10 μm.—8. Longitudinal section of a young ovule with an enlarged megaspore mother cell (*mc*). Note the position of a nucleus. Bar = 10 μm.—9. Longitudinal section of a young ovule with a dyad composed of a smaller micropylar megaspore (*c*) and a larger

date the equally unusual but not directly related *Alzatea.*

Until we recently reported on the embryology of *Axinandra* (Tobe & Raven, 1983b), no embryological information was available on the genera that were relegated to Crypteroniaceae sensu lato by van Beusekom-Osinga and van Beusekom (1975). On the basis of available embryological information, however, we cannot evaluate the proposed inclusion of *Axinandra* in Crypteroniaceae together with *Crypteronia* and *Dactylocladus,* since the latter two genera are unknown in this respect. The purpose of the present paper is to evaluate the relationship of *Rhynchocalyx* to *Axinandra,* Lythraceae, and other Myrtales, and in the light of this information to comment upon its phylogenetic relationships and proper taxonomic placement.

Material and Methods

Flower buds and fruits of *Rhynchocalyx lawsonioides* Oliv. were collected and fixed in FAA (five parts stock formalin: five parts glacial acetic acid: 90 parts 70% ethanol) at Uvongo River, Natal, Republic of South Africa, by Mr. H. B. Nicholson. The voucher specimen is *Nicholson s.n.,* in 1982 (MO). Preparations of microtome sections for the observation were made following the techniques discussed in a previous paper (Tobe & Raven, 1983b).

Observations

Anther and Microspores

The anther is tetrasporangiate. The wall structure prior to maturation comprises five layers, i.e., an epidermis, an endothecium, two middle layers, and a tapetum (Fig. 1). Since the outer and the inner middle layers share their developmental origins with the endothecium and the tapetum, wall formation conforms to the Basic type (Davis, 1966: 10). During the process of maturation, the epidermis remains uncrushed whereas the endothecium and the two middle layers degenerate (Fig. 2). The tapetum is glandular and its cells become two-nucleate before degeneration. Thus a mature anther wall at the time of dehiscence is composed only of the persistent epidermis (Figs. 3, 4). The outer half of each epidermal cell forms a conspicuous papilla (Fig. 3). A septum between two microsporangia on each side of the anther remains intact even at the time of anther dehiscence (arrows, Fig. 4), a condition that is unusual in angiosperms.

Meiosis in a microspore mother cell is accompanied by simultaneous cytokinesis. Shape of the resultant microspore tetrads is, on the basis of the examination of 20 selected tetrads, "usually" (65%) tetrahedral and "often" (35%) decussate (expressions of the frequency follow Schmid, 1982). Pollen grains are two-celled when they are shed (Fig. 5).

Megagametophyte and Nucellus

The ovule is anatropous and crassinucellate. The archesporium is hypodermal and is composed of three to five cells, only one of which further divides periclinally into two: i.e., the upper primary parietal cell and the lower sporogenous cell (Fig. 6). The primary parietal cell divides once anticlinally (Fig. 7) and both cells repeat periclinal divisions to form a massive parietal tissue. The sporogenous cell develops into a megaspore mother cell (Fig. 7) and becomes enlarged by the time of meiosis (Fig. 8). In the enlarged megaspore mother cell a nucleus occupies a position on the micropylar side (Fig. 8). Meiosis in the megaspore mother cell is accompanied by successive cytokinesis. By the first meiotic division, the megaspore mother cell is partitioned into a dyad of megaspores in which the micropylar cell is much smaller than the chalazal cell (Fig. 9). The second meiotic division occurs earlier in the micropylar cell of the dyad than in the chalazal cell. The two daughter cells thus formed on the micropylar side are always

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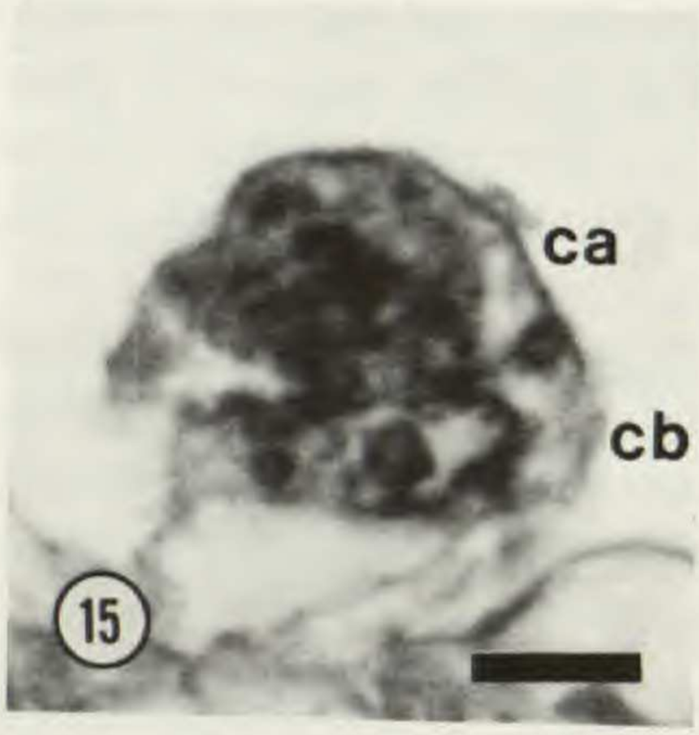
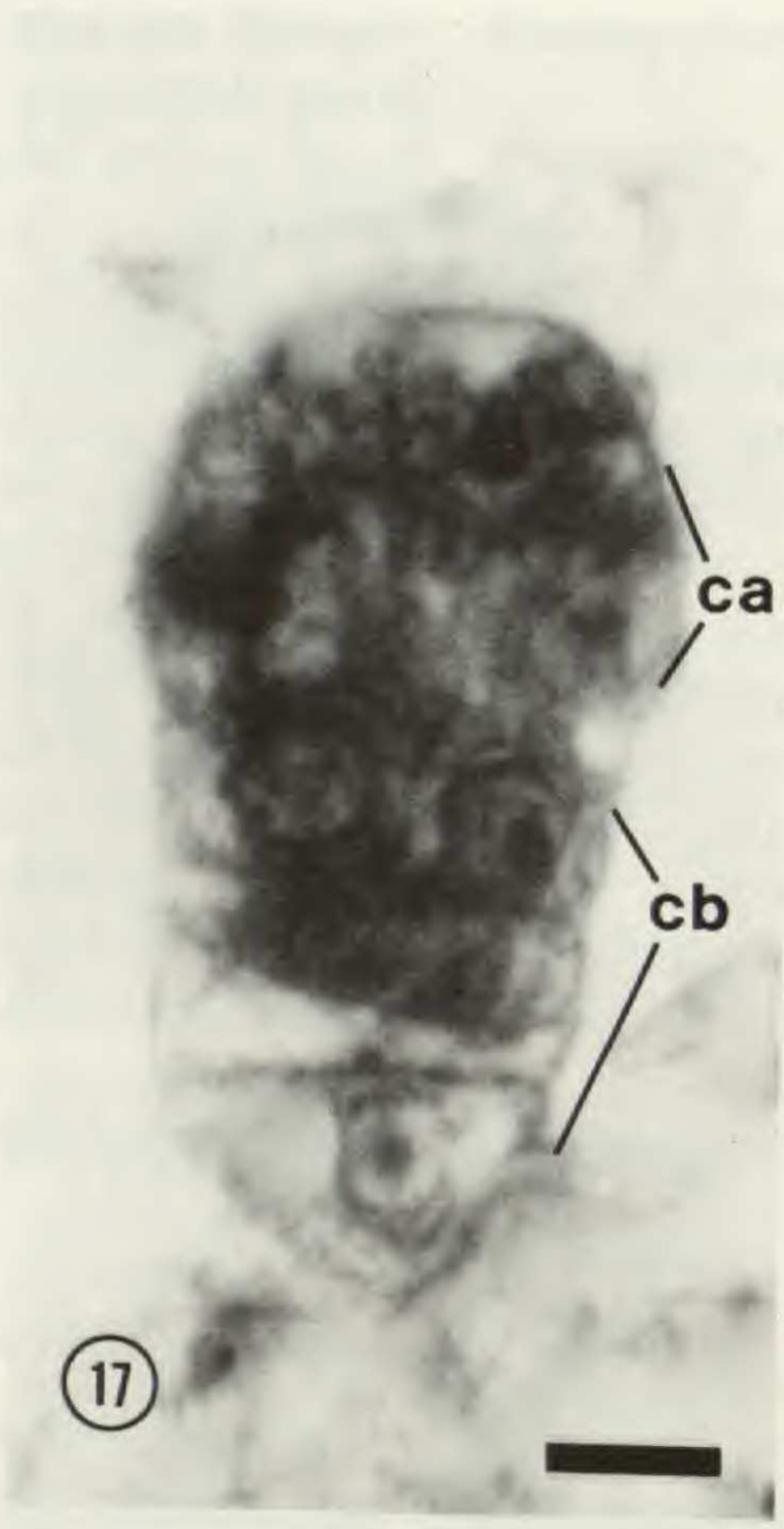
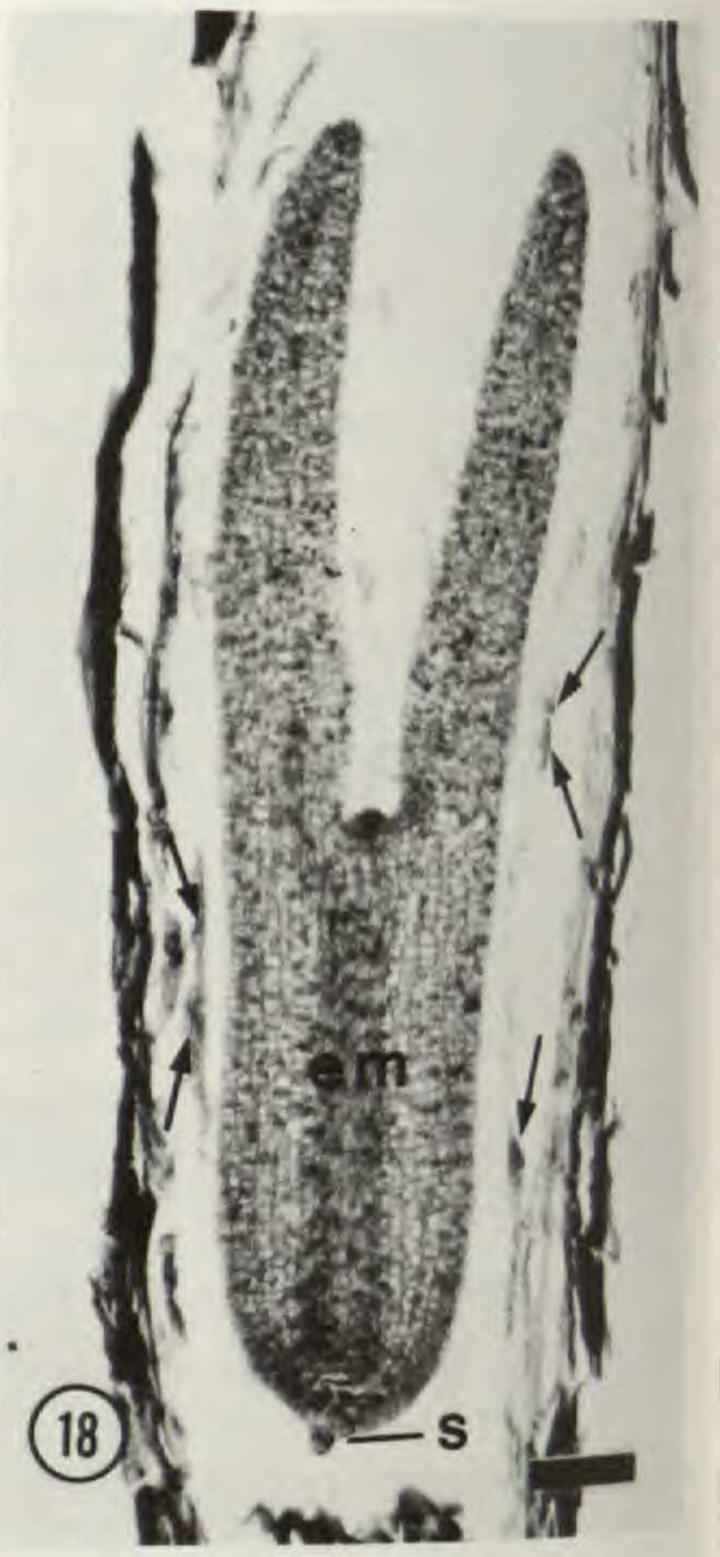
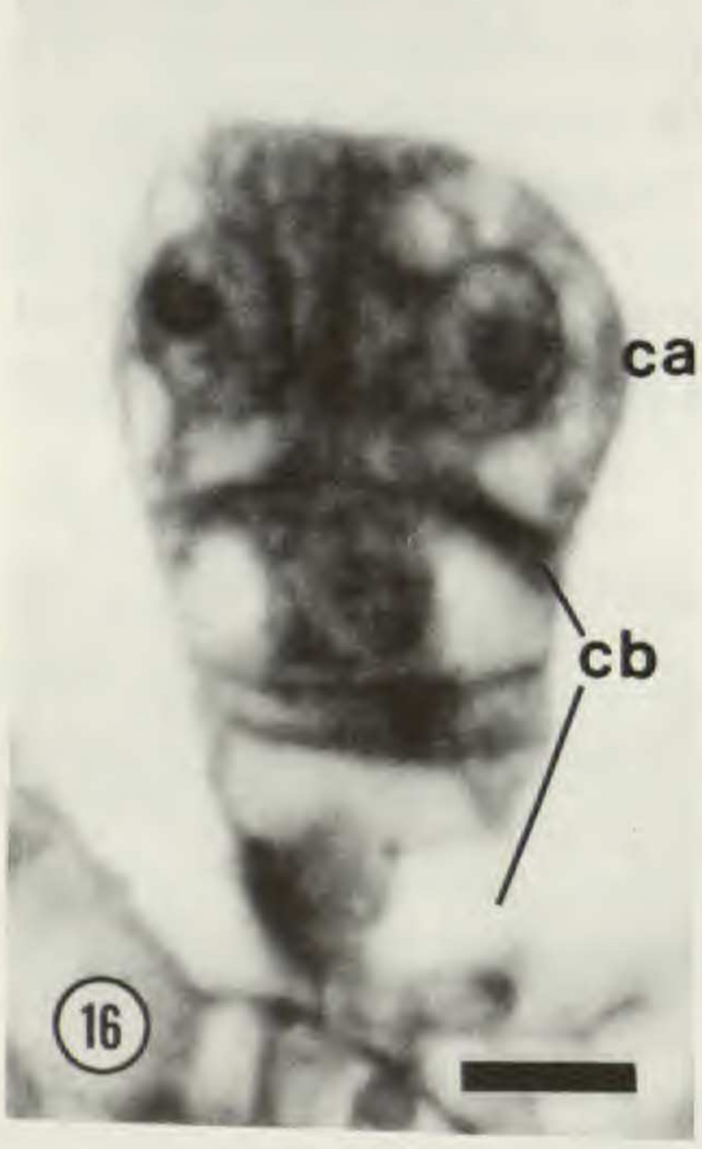
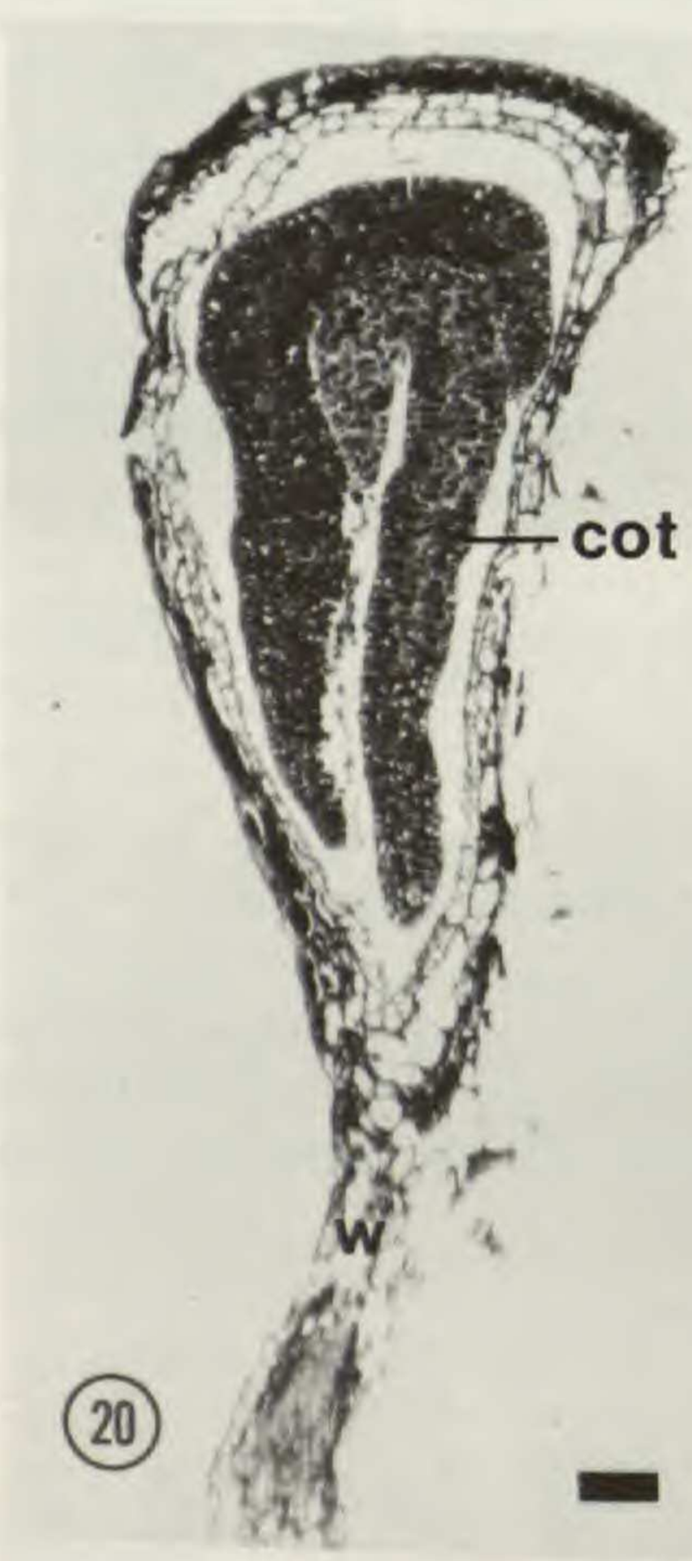
chalazal megaspore (*c*). Bar = 10 μm.—10. Longitudinal section of a young ovule with a linear tetrad of megaspores (*c*). Note that the two micropylar megaspores are already crushed while the two chalazal megaspores are being formed. Bar = 10 μm.—11. Longitudinal section of a young ovule with a functional chalazal megaspore enlarging (second *c* from bottom). Note that subdermal cells of the nucellus are radially elongated. Bar = 10 μm.—12. Longitudinal section of a mature embryo sac with degenerating antipodals (arrow). Bar = 10 μm.—13. Longitudinal section of a micropylar part of a mature ovule. Note the two-layered structure of the inner (*ii*) and the outer (*oi*) integument. A micropyle (*m*) is formed by the inner integument alone. *eg*: egg cell; *sy*: synergid. Bar = 10 μm.—14. Longitudinal section of a mature ovule to show the whole structure. Note the position wing (*w*) formed by the funicular tissue. *ii*: inner integument; *oi*: outer integument; *m*: micropyle. Bar = 100 μm.

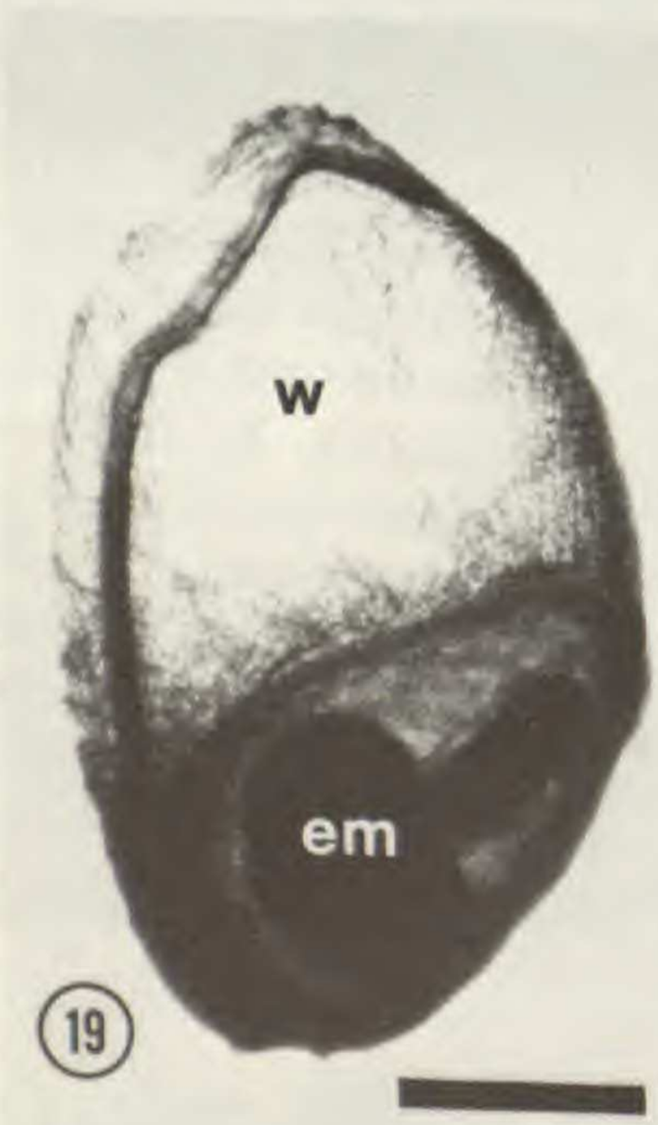












FIGURES 15–21. —15. Longitudinal section of a two-celled proembryo composed of an apical cell (*ca*) and a basal cell (*cb*). The apical cell is dividing vertically. Bar = 10 μm.—16. Longitudinal section of an eight-celled proembryo. *ca*: cells derived from the apical cell at the two-celled stage; *cb*: cells derived from the basal cell. Bar = 10 μm.—17. Longitudinal section of an older proembryo. Note that cells derived from the apical cell (*ca*) form a global part of the proembryo whereas those derived from the basal cell (*cb*) form the lower rod. Bar = 10 μm.—18. Longitudinal section of a young seed including a young dicotyledonous embryo (*em*). Arrows indicate free endosperm nuclei. *s*: suspensor. Bar = 100 μm.—19. Nearly mature seed with a membranous wing

degenerating when the chalazal cell of the dyad is dividing (Fig. 10). A cell plate in the division of the chalazal cell of the dyad is also formed on the more or less micropylar side (Figs. 10, 11). Megaspore tetrads are always linear (Fig. 11) although the upper (i.e., micropylar) two are often arranged somewhat obliquely, and the lowest (chalazal) cell functions and thus develops into an eight-nucleate *Polygonum*-type embryo sac. Synergids are pyriform, and three antipodals are ephemeral; they degenerate without any sign of wall formation (arrow, Fig. 12). Consequently, an organized mature embryo sac just before fertilization comprises only five nuclei or cells—i.e., an egg, two synergids, and two polar nuclei.

During megasporogenesis, subdermal cells of the nucellus elongate radially and form a jacket around the enlarged megaspore mother cell or the megaspores (Figs. 8–11). This tissue, however, gradually degenerates or is absorbed as the embryo sac enlarges in the later stages.

INTEGUMENTS

The ovule is bitegmic (Figs. 13, 14). Both the inner and the outer integument are initiated by periclinal divisions of dermal cells of the nucellus and grow only by divisions of the cells derived from the dermal initial cells, resulting in a two-layered structure. Neither of the integuments show any secondary multiplication in thickness (Figs. 13, 14). Cells of the outer epidermis of the outer integument become tanniferous as early as the megaspore mother cell stage.

The inner integument exceeds the outer integument in its degree of elongation (Figs. 13, 14); thus the micropyle is always formed by the inner integument alone (Figs. 13, 14).

FERTILIZATION, ENDOSPERM, AND EMBRYO

Fertilization is porogamous. Endosperm formation is of the Nuclear type. The endosperm is very scanty even at the free nuclear stage and throughout the process of seed development (arrows, Fig. 18); it does not show any particular accumulations of free nuclei on the micropylar or on the chalazal side. Wall formation does not occur in free nuclei. We could not find cellular endosperm at any stage of seed development. The mature seeds completely lack endosperm (Figs. 20, 21).

Embryogenesis conforms to the Onagrad type. The apical cell of a two-celled proembryo divides vertically, and the basal cell transversely (Fig. 15). Cells derived from the apical cell contribute to the formation of a major part of the embryo, those derived from the basal cell only to a minor portion. The latter include the parts that are destined to form the root cap and cortex, as well as the suspensor (Figs. 16, 17). A young embryo has two equally developed cotyledons and a short and small suspensor (Fig. 18). The embryo in the mature seed is more or less flattened, its cotyledons folded inside (Fig. 20).

MATURE SEED AND SEED COAT

The mature seed is depressed-ovoid in shape with a flat membranous wing on the micropylar side (Fig. 19). The wing is formed by divisions and elongation of cells of the funiculus (Figs. 14, 19). No hypostase is formed throughout the development of ovule and seed.

The mature seed coat is derived from the two-layered testa as well as from the two-layered tegmen. All of the constituent cells are highly elongated, particularly longitudinally. The outer surface of the outer epidermis of the testa is conspicuously lignified.

DISCUSSION

The embryological characteristics of *Rhynchocalyx lawsonioides* may be summarized as follows:

Anther tetrasporangiate; anther wall five layers thick, its formation of the Basic type; anther epidermis persistent, the outer half part of each cell forming a conspicuous papilla; endothecium and two middle layers ephemeral; tapetum glandular, its cells two-nucleate; septum between the two microsporangia on each side of the anther persistent. Cytokinesis in microspore mother cells simultaneous; microspore tetrads tetrahedral or decussate in shape; pollen grains two-celled when shed.

Ovule anatropous, bitegmic, and crassinucel-

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(*w*). *em*: embryo. Bar = 1 mm.—20. Cross section of a mature seed. Cotyledons (*cot*) of the embryo are folded inside. *w*: wing. Bar = 100 μm.—21. Longitudinal section of a mature seed. Both the two-layered testa (*ts*) and the two-layered tegmen (*tg*) constitute a mature seed coat. *em*: embryo. Bar = 100 μm.

late, both integuments two-layered; subdermal cells of the nucellus elongated radially, forming a jacket around the megaspore mother cell or megaspores; micropyle formed by the inner integument alone; chalaza without hypostase.

Archesporium of ovule multicelled, comprising three to five cells, only one of them functioning and cutting off a parietal cell; cytokinesis at meiosis I resulting in a dyad composed of a smaller micropylar cell and a larger chalazal cell; the subsequent division of meiosis II earlier in the micropylar cell of the dyad than in the chalazal cell; tetrads of megaspores linear; chalazal megaspore functional, developing into an eight-nucleate *Polygonum*-type embryo sac; antipodals ephemeral.

Fertilization porogamous; endosperm formation of the Nuclear type; wall formation not occurring in free endosperm nuclei; embryogenesis conforming to the Onagrad type; embryo dicotyledonous with a short and small suspensor; seed exalbuminous with a single flat membranous wing on the micropylar side. Seed coat thin and fibrous, consisting of elongate cells of the two-layered testa as well as of the two-layered tegmen.

Rhynchocalyx shares six of the seven basic embryological criteria that define the order Myrtales (Tobe & Raven, 1983a): (1) anther tapetum glandular, (2) ovule crassinucellate, (3) inner integument two-layered, (4) antipodals ephemeral, (5) endosperm formation the Nuclear type, and (6) mature seed exalbuminous (cf. Tobe & Raven, 1983a). The only disagreement is that *Rhynchocalyx* has a micropyle formed by the inner integument alone instead of by both integuments, and this seems to be a feature subject to evolutionary modification within the group, indicating that the situation in *Rhynchocalyx* is a derived one.

As regards the view of van Beusekom-Osinga and van Beusekom (1975) that *Rhynchocalyx* should be assigned to Crypteroniaceae sensu lato, we still have embryological information only concerning *Axinandra*, but lack it for the other constituent genera. *Rhynchocalyx* agrees with *Axinandra* in having an ephemeral endothecium (which is also characteristic of the Melastomataceae as a whole), but differs from *Axinandra* in many features. In *Rhynchocalyx*, the septum between two microsporangia on each side of the anther is persistent; the archesporium is multicelled; the micropyle is formed by the inner integument alone; the endothelium is not formed; the seed wing is formed on the micropylar side. In contrast, in *Axinandra* the septum in the anther is ephemeral; the archesporium is one-celled; the micropyle is formed by both integuments; the endothelium is formed; the seed wing is formed on the chalazal side. Evidence from the embryology, as well as from vegetative anatomy (van Vliet, 1975; van Vliet & Baas, 1975), indicates the heterogeneity of Crypteroniaceae sensu lato and virtually excludes the possibility that it would be desirable to retain both *Rhynchocalyx* and *Axinandra* in the same family.

As regards the alternative view that *Rhynchocalyx* should be (re-)placed in Lythraceae (van Vliet, 1975; van Vliet & Baas, 1975), the presence of the multicelled archesporium in an ovule supports the view because this characteristic has been found only in Lythraceae (including Soneratiaceae). Contradicting the view that *Rhynchocalyx* might be directly related to Lythraceae, however, are the facts that *Rhynchocalyx* has an ephemeral endothecium and a micropyle formed by the inner integument alone, both features that have not been reported in Lythraceae. In fact, in its ephemeral endothecium, *Rhynchocalyx* agrees with *Axinandra* and Melastomataceae rather than with Lythraceae. The micropyle difference constitutes a distinct gap between *Rhynchocalyx* and Lythraceae. Some additional points of difference in embryology between *Rhynchocalyx* and Lythraceae include the following. In *Rhynchocalyx*, the septum between two microsporangia on each side of the anther is persistent; nucellar subdermal cells elongate radially, forming a jacket around the megaspores; the homotypic division of meiosis occurs earlier in the micropylar cell of the dyad than in the chalazal cell; starch grains are absent in the nucellus; and the endosperm is too scanty to show accumulations of free nuclei in the embryo sac. In Lythraceae, on the other hand, the septum in the anther probably collapses, as is the case in most angiosperms; radially elongated nucellar subdermal cells have not been observed; the homotypic division occurs later in the micropylar cell of the dyad than in the chalazal cell; starch grains are present in the nucellus (*Cuphea*, Hubert, 1896); and the endosperm commonly shows accumulations of free nuclei on the micropylar and/or the chalazal region of the embryo sac (Joshi & Venkateswarlu, 1936) and may become cellular (Mauritzon, 1934).

In view of all these points of difference, we conclude that embryology provides strong support for the conclusion of Johnson and Briggs

(1984) that *Rhynchocalyx* should be assigned to a monotypic family not directly related to Lythraceae. *Rhynchocalyx* does share its distinctive archesporial characteristics with Lythraceae, but significant gaps between these two taxa are evident in the characters of the anther wall, micropyle, nucellus, megasporogenesis, and endosperm. There is no strong evidence linking this remarkable South African relict genus directly with any other group, and it seems clear that it is best placed in a family of its own in order to emphasize its great distinctiveness.

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